Effect of casein to whey protein ratios on the protein interactions and coagulation properties of low-fat yogurt

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ABSTRACT

In this study, we investigated the effect of casein (CN) to whey protein (WP) ratios (4:1, 3:1, 2:1, and 1:1) on gelation properties and microstructure of low-fat yogurt made with reconstituted skim milk with or without addition of whey protein concentrate. The rheological properties (storage modulus, G' ; yield stress; and yield strain) of the obtained low-fat yogurt were greatly enhanced, the fermentation period was shortened, and the microstructure became more compact with smaller pores as the CN:WP ratio decreased. When CN:WP was 2:1 or 1:1, the obtained yogurt coagulum showed higher G' and greater yield stress, with more compact crosslinking and smaller pores. In addition, the more of skim milk powder was replaced by whey protein concentrate, the more disulfide bonds were formed and the greater the occurrence of hydrophobic interactions during heat treatment, which can improve the rheological properties and microstructure of low-fat yogurt.

Key words: casein to whey protein ratio, low-fat yogurt, rheological behavior, microstructure

INTRODUCTION

Low-fat yogurt has been attracting increasing attention from health-conscious consumers because of its nutritional values and health-promoting properties (Küçükçetin, 2008). However, milk fat has important roles in emulsification and in flavor and texture development in the formed gels. A reduced fat content may reduce viscosity and increase whey syneresis, which can affect the appearance, texture, and mouthfeel of low-fat yogurt (Lee and Lucey, 2004; Houzé et al., 2005). Therefore, improving the structure and flavor deficiencies of low-fat yogurt deserves close attention.

Several methods have been used to improve the properties of low-fat yogurt, including addition of whey protein (WP), use of suitable starter cultures, enhanced total solids content, use of thickeners, and modification of processing parameters (Sodini et al., 2004). The viscoelasticity or apparent viscosity of yogurt can be increased 2 to 3 times by adjusting the total solids and protein contents or by adding thickeners or enzymes to the milk base. Addition of thickeners (polysaccharides or gelatin) or enzymes (lactoperoxidase, protease, and transglutaminase) allows for new cross-links in the network and enhances the rigidity of the gel and its water-holding capacity. Processing parameters, including heat treatment, shearing, homogenization, and storage period, affect texture and thickness-in-mouth of yogurt. Starter cultures help to improve the smoothness and water-holding capacity of yogurt, because they can produce exopolysaccharides (EPS) during fermentation and gel formation. The addition of increasing levels of β -LG causes marked increases in storage modulus (G') compared with α -LA, and some differences in behavior exist among the different β -LG variants (Graveland-Bikker and Anema, 2003).

Heat treatment, one of the predominant processes of dairy product manufacturing, leads to denaturation of milk proteins and interaction among denatured milk proteins, which may dramatically affect the texture and consistency of yogurt (Mulvihill and Grufferty, 1995). Whey proteins are much more heat-sensitive than casein. The denatured WP interact with each other to form soluble WP aggregates or interact with casein micelles to form WP-coated casein micelles (Krzyminski et al., 2011). Most WP are denatured during normal heat treatment of yogurt manufacture, during which the formation of disulfide bonds and occurrence of hydrophobic interactions within denatured WP and between denatured WP and κ -CN on the surface of casein micelles leads to the formation of WP- κ -casein complexes (Smits and Brouwershaven, 1980; Haque and Kinsella, 1988; Singh and Myhr, 1998). Addition of WP has potential application in the manufacture of yogurt

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(Lucey et al., 1999; Graveland-Bikker and Anema 2003), cheese (Hinrichs, 2001; Kelly and O’Kennedy, 2001), and other coagulated dairy products; it not only improves physical properties and microstructure but also alters the functional properties of the obtained products. Therefore, heat treatment of milk fortified with WP is conducive to a high level of crosslinking within the gel network, which results in a denser yogurt structure and enhanced yogurt viscosity and water-holding capacity (Remeuf et al., 2003).

Although WP fortification has been investigated in the processing of low-fat yogurt, it is difficult to independently evaluate the effect of whey proteins on yogurt properties because the addition of WP also changes the protein and TS contents (Damin et al., 2009). Including a high proportion of WP might impart an undesirable whey flavor as well as a grainy texture under some conditions (Lucey and Singh, 1997; González-Martínez et al., 2002). In addition, a relatively low content of casein is believed to result in a more open gel structure, making the coagulum network more sensitive to syneresis (González-Martínez et al., 2002). Therefore, determining the proper ratio of CN to WP is critically important. Whey protein concentrate (WPC) had been used to partially substitute for skim milk powder (SMP) to alter the CN:WP ratio in yogurt manufacture (Bhullar et al., 2002; Akalin et al., 2008). By using a decreased CN:WP ratios, increased maximum gel strength, reduced whey drainage, and a denser network could be obtained (Puvanenthiran et al., 2002; Küçükçetin, 2008), and lower viscosity and higher intensities of graininess and yellow color would be also exhibited (Tomaschunas et al., 2012). Although some research has been conducted on CN:WP ratios in yogurt, the effects on the fermentation process and the interaction between CN and WP have not yet been investigated.

The objective of this research was to investigate the effect of CN:WP ratios with constant protein content on the gelation properties and microstructure of low-fat yogurt. The interactions within denatured whey protein

and casein micelles was also evaluated by determination of disulfide bonds and surface hydrophobicity. Samples with CN:WP ratios of 3:1, 2:1, and 1:1 were compared with a reference yogurt manufactured with skim milk powder with a CN:WP ratio of 4:1.

MATERIALS AND METHODS

Materials and Chemicals

Skim milk powder (by weight, 32.5% protein, 0.43% fat, 52.1% carbohydrates; CN:WP = 4:1) and whey protein concentrate 80 (WPC80; by weight, 81.23% protein, 0.11% fat, 13.30% carbohydrates) were supplied by Hilmar Ingredients (Hilmar, CA). 8-Anilino-1-naphthalenesulfonic acid (ANS) and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents used in the present study were of analytical grade.

Preparation of Low-Fat Yogurt

Milk Base Preparation. Control milk base was prepared as follows: 12.94 g of SMP was added to 87.06 g of distilled water at 25°C (CN:WP ratio = 4:1). Experimental trials were prepared by mixing SMP with WPC80 quantitatively at CN:WP ratios of 3:1, 2:1, and 1:1, respectively, as shown in Table 1. The total milk base content was 100 g and the protein content of all samples was 4% (wt/wt). The milk bases were stirred for 3 h at 25°C and stored at 4°C overnight to ensure complete hydration.

Fermentation. One hundred grams of each sample was prepared. After homogenization at 20 MPa and 55°C, each milk base was treated in a thermostatically controlled boiling water bath (DK-8B, Jinghong Laboratory Equipment Co. Ltd., Shanghai, China) until the center temperature of sample reached 95°C and was then held for 5 min. The heated samples were immediately cooled to 42°C for analysis and further fermentation. Direct Vat Set starter culture (ABY-8, Chr. Hansen, Milwaukee, WI) containing *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Bifidobacterium* was added at the recommended concentration of 0.005% (wt/wt). Milk samples were incubated at 42°C, and the fermentation process was immediately stopped by rapidly cooling to 4°C in an ice-water bath until the pH value decreased to 4.60. The obtained yogurt samples were stored at 4°C for further measurements. The samples were prepared in triplicates and new preparation was made for each replicate.

Table 1. The formula of milk base with different casein to whey protein ratios

Item ¹	Casein to whey protein ratio			
	4:1	3:1	2:1	1:1
SMP (g/100 mL)	12.94	12.13	10.78	8.08
WPC (g/100 mL)	0	0.33	0.87	1.96
Total protein (%)	4.0	4.0	4.0	4.0
Total solids (%)	12.94	12.46	11.65	10.04

¹SMP = skim milk protein; WPC = whey protein concentrate.

Determination of Rheological Properties

Yogurt gel formation process was monitored using a Universal Dynamic Spectrometer (AR1500ex, TA Instruments, New Castle, DE) with a top plate (diameter 60 mm). Two-milliliter milk samples, which were inoculated with starter culture, were transferred to the bottom. A few drops of silicone oil were added to cover the edge of the plate to prevent evaporation during the measurements. The oscillation was set at a constant frequency of 1 Hz and a constant strain of 0.1%, which caused minimal disruption during the development of gel network (Van Camp et al., 1997). Measuring points were taken every 1 min until pH 4.6 was reached. Gelation time was defined as the moment when the G' values of the obtained gels were greater than 1 Pa (Lucey et al., 1997). Fermentation time was the time for pH to reach 4.6. Two samples were prepared at the same time, one for monitoring pH and fermentation time and the other for determination of rheological properties.

The large-deformation properties of yogurt gels were determined by applying a single constant shear rate ($\sim 0.01 \text{ s}^{-1}$). The strain applied varied from 0.003 to 100%. Yield stress (σ_{yield}) was defined as the point when shear stress started to decrease, and yield strain (y_{strain}) was the strain value at the yield point (Lucey, 2002).

Microstructure Measurement

Confocal scanning laser microscopy was used to evaluate the microstructure of yogurt gels as reported by Lucey et al. (1998). Rhodamine B (Sigma Chemical Co.) was used as a fluorescent protein dye and dissolved in demineralized water to a concentration of 10 mg/mL. One milliliter of Rhodamine B solution and starter culture was added to 100 g of heat-treated milk and mixed using a magnetic stirrer (CJJ78-1, Jingke Scientific Instrument Co. Ltd., Shanghai, China) for 5 min. A few drops of each sample was transferred to a concave slide with a coverslip, which were then incubated at 42°C until the pH value reached 4.6. The end-point of fermentation was judged using the remainder of the 100-g milk samples that were fermented at the same time. The obtained gel samples were observed using a Leica TCS 4D confocal microscope (Leica Laser-technik GmbH, Heidelberg, Germany) with a 60 $\times$ oil immersion objective (numerical aperture = 1.4) at an excitation wavelength of 568 nm. All experiments were done in triplicate. Many fields were viewed and typical micrographs are presented.

Determination of Disulfide Bond Content

Disulfide bond (-S-S-) contents of the samples were determined using Ellman's reagent according to the

method described by Beveridge et al. (1974). First, 0.2 mL of heated or unheated milk dispersion was diluted with 1 mL of buffer (0.086 M Tris, 0.09 M glycine, 4 mM EDTA, 10 M urea, pH 8.0), 0.02 mL of β -mercaptoethanol, and 10 mL of 12% trichloroacetic acid (TCA); incubated for 1 h at 25°C; and then centrifuged at $5,000 \times g$ for 15 min. The precipitate was washed twice with 12% (wt/vol) TCA and centrifuged as described above. The obtained sediment was subsequently dissolved in 4 mL of standard buffer (0.086 M Tris, 0.09 M glycine, 4 mM EDTA, pH 8.0), 0.03 mL of Ellman's reagent was added, and the mixture was held for 10 min, and the absorbance at 412 nm was measured by a spectrophotometer (UV-2600, Unicop Instrument Co. Ltd., Shanghai, China). The disulfide bond content was calculated using a molar extinction coefficient of $13,600 \text{ M}^{-1}\cdot\text{cm}^{-1}$ (Ellman, 1959) and expressed as micromoles per gram of protein.

Determination of Surface Hydrophobicity

Surface hydrophobicity was determined according to the method described by Alizadeh-Pasdar and Li-Chan (2000). Milk samples were diluted at 5 protein concentrations between 0.0025 and 0.0125% by addition of PBS (0.01 M, pH 7.0). Then, 40 μL of 8 mM ANS buffer was added to 4 mL of diluted milk dispersion. The relative fluorescence intensity of the samples was measured using a fluorescence spectrophotometer (model RF-5301PC, Shimadzu, Kyoto, Japan), with excitation and emission wavelength of 390 and 470 nm, respectively. Samples without ANS addition were recorded as blanks. Surface hydrophobicity was calculated according to the slope method and expressed as the specific value of initial slope of the relative intensity and protein concentration.

Statistical Analysis

The experimental design was a single factor design and all experiments were carried out at least 3 times with 3 replicates in this study. Data are reported as means $\pm$ standard deviations (SD). Analysis of significant difference and variance tests were performed by one-way ANOVA using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL). Differences were considered significant at $P < 0.05$.

RESULTS

Coagulation Properties of Low-Fat Yogurt Gels with Different CN:WP Ratios

The rheological properties of low-fat yogurt fortified with different CN:WP ratios were observed in the for-

mation process. The G' profiles as a function of pH of yogurts are shown in Figure 1. When the pH value was >5.83 , the G' profiles of the yogurt samples were similar and close to zero. When pH values were between 5.76 and 5.27, the G' value of yogurt with CN:WP of 1:1 increased continuously, whereas profiles of the other 3 samples exhibited a shoulder. When pH was <5.27 , the G' values of all samples increased until the end of fermentation process, whereas the rate of increase of G' was the highest in yogurt with a CN:WP ratio of 1:1, which correspondingly had the highest G' value at pH 4.6.

Rheological Properties of Low-Fat Yogurts with Different CN:WP Ratios

Rheological properties of low-fat yogurt prepared at CN:WP ratios from 4:1 to 1:1 are presented in Table 2. The decrease in CN:WP ratios with constant total protein content considerably shortened the fermentation time and gelation time of yogurt. The G' values at pH 4.6 and pH values at gelation increased when the CN:WP ratio decreased. When the CN:WP ratio was 1:1, fermentation time and gelation time were the shortest, whereas the pH at gelation time point and G' at pH 4.6 were the highest.

The results of the large-deformation test are presented in Figure 2. A decrease in CN:WP ratio resulted in an increase in yield stress and strain of low-fat yogurt. The sample with a CN:WP of 1:1 had the highest yield stress and strain, 54 Pa and 42.81%, respectively, whereas the sample with a CN:WP of 4:1 had the lowest yield stress and strain values, 4.2 Pa and 12.28%, respectively.

Microstructure of Low-Fat Yogurts with Different CN:WP Ratios

The microstructures of low-fat yogurt gels with different CN:WP ratios were examined by confocal scanning laser microscopy as shown in Figure 3. At a CN:WP of 4:1, the low-fat yogurt showed a more interrupted structure with larger pores (diameter ~ 20

Figure 1. The change of storage modulus (G') as a function of pH for low-fat yogurts fortified with casein to whey protein ratios of 4:1 (control), 3:1, 2:1, and 1:1.

μm ; Figure 3A). As the CN:WP ratio decreased, the crosslinked network became denser and finer, and the pores became smaller, as shown in Figure 3B, C, D. The yogurt with a CN:WP of 1:1 (Figure 3D) appeared the most homogeneous, with the smallest and most evenly distributed pores.

Effect of CN:WP and Temperature on Disulfide Bond Content and Surface Hydrophobicity

The disulfide bond contents of samples are presented in Table 3. Disulfide bond contents increased dramatically after heat treatment and increased with decreasing CN:WP ratio ($P < 0.05$). The disulfide bond content reached a maximum of $27.78 \pm 1.24 \mu\text{mol/g}$ of protein at 95°C when the CN:WP ratio was 1:1.

The surface hydrophobicity (S_0) of samples was estimated using ANS as a fluorescence probe, with data shown in Table 4. When the protein molecule was un-

Table 2. Rheological properties (means $\pm$ SD) of low-fat yogurts fortified with different casein to whey protein ratios

Item	Casein to whey protein ratio			
	4:1 (Control)	3:1	2:1	1:1
Fermentation time (min)	270.12 $\pm$ 2.31 ^a	262.22 $\pm$ 4.43 ^b	251.23 $\pm$ 5.82 ^c	240.15 $\pm$ 3.54 ^d
Gelation time (min)	96.56 $\pm$ 4.62 ^a	88.42 $\pm$ 3.21 ^b	81.22 $\pm$ 3.11 ^c	76.12 $\pm$ 1.24 ^d
pH at gelation	5.71 $\pm$ 0.01 ^d	5.78 $\pm$ 0.02 ^c	5.82 $\pm$ 0.01 ^b	5.88 $\pm$ 0.01 ^a
G' (storage modulus) at pH 4.6 (Pa)	40.76 $\pm$ 5.23 ^d	51.12 $\pm$ 4.21 ^c	118.82 $\pm$ 13.23 ^b	206.35 $\pm$ 23.45 ^a

^{a-d}Means within a row with different letters are significantly different ($P < 0.05$).

Figure 2. Shear stress as a function of strain applied deformation at a constant shear rate (0.01/s) for low-fat yogurts fortified with casein to whey protein ratios of 4:1 (control), 3:1, 2:1, and 1:1.

folded and the inner hydrophobic groups reacted with the ANS probe, an increase of fluorescence intensity was expected and the change of S_0 values was observed. The S_0 values of samples at 95°C increased significantly with decreasing CN:WP ratios ($P < 0.05$), whereas the opposite tendency ($P < 0.05$) of S_0 values was observed at 25°C. The S_0 values at 95°C were higher than those at 25°C. Thus, S_0 was affected not only by heat intensity but also by CN:WP ratio.

DISCUSSION

The present study showed that when the CN:WP ratio was decreased to 2:1 and 1:1 with the addition of WPC80, the low-fat yogurt possessed higher values of G' and yield stress and a more cross-linked network structure with smaller pores (Figures 1 to 3). These results were consistent with Küçükçetin (2008) and Puvanenthiran et al. (2002), who found that increased G' and yield stress and a denser microstructure could be gained when the CN:WP ratio was decreased or the heating temperature was increased. This finding may be due to the shift in the amount of denatured whey proteins, which could be aggregated to provide linking protein agents (Lucey et al., 1999). These linking protein agents could interact with caseins to form whey protein-coated casein micelles. Increasing the amount of casein-whey complexes and connecting points could lead to increased branching and, consequently, gels

with high G' and firmness (Lucey et al., 1997). A higher volume fraction of whey proteins could lead to an increased number of covalent disulfide bonds that play a more important role than noncovalent bonds in gel structure during network formation (Krzeminski et al., 2011). Large particles could be formed by the addition of whey protein, which enables the gels to withstand by interparticle flexing forces and exhibit a high yield stress, without breaking the intraparticle cross-link bonds. A “shoulder” was observed for yogurts with CN:WP ratios of 4:1, 3:1, and 2:1, as shown in Figure 1, which might be due to the solubilization of colloidal casein phosphopeptides (CCP) from micelles, resulting in a transient loosening of the gel network (Anema, 2009). The CCP was solubilized when gelation occurred at a higher pH, which weakened the internal structure of gel at pH 5.6 to 5.4. The electric charges of proteins decreased as the pH continued to decrease, which made the proteins interact more strongly, and the G' increased again at lower pH. Therefore, the G' shoulder was increased when the CCP level was increased in the sample with CN:WP ratios of 4:1, 3:1, and 2:1, which possessed higher casein content. This G' shoulder phenomenon was also found by Andoyo et al. (2015) and Anema (2009).

Samples enriched with WPC80 also showed a short gelation time and a high gelation pH (Table 1). During yogurt fermentation, the buffering compounds in milk, such as soluble calcium phosphate and basic AA side chains of casein, become protonated and the micellar calcium phosphate dissolves in the serum phase (Gaucheron, 2005). A decreased concentration of buffering compounds from casein would decrease the buffering capacity and consequently accelerate the fermentation process. Moreover, the lower the CN:WP ratio, the greater the opportunity for whey to aggregate with κ -casein on the surface of micelles, which might also explain the shorter gelation time. However, Puvanenthiran et al. (2002) found that fermentation time increased when the CN:WP ratio was decreased by WPC40 addition, which was opposite to the behavior observed in the present study. These results demonstrate that different whey protein concentrates (WPC80 and WPC40) with different compositions might be the major factor affecting the length of fermentation. The present study showed that the pH value at gelation time was higher when the proportion of whey proteins in milk base increased, which was most probably due to the increased participation of denatured whey proteins that had generally higher isoelectric points ($pI \approx 5.3$ for β -LG) than casein proteins. When the pH of the milk base was reduced and approached the isoelectric point of the unfolded and aggregated whey proteins, yogurt gels started to form (Jørgensen et al., 2015).

Figure 3. Electron micrographs of low-fat yogurts produced with casein to whey protein (CN:WP) ratios of 4:1 (Control, A), 3:1 (B), 2:1 (C), and 1:1 (D). As the CN:WP ratio decreased, the crosslinked network became denser and finer, and the pores became smaller, as shown in panels B, C, and D. The yogurt with a CN:WP of 1:1 (panel D) appeared the most homogeneous, with the smallest and most evenly distributed pores. Color version available online.

O’Kennedy and Kelly (2000) obtained a similar result and suggested that interactions between caseins and heat-denatured whey proteins could occur at pH 5.8 to 6.2. However, a lower gelation pH value of 5.25 was obtained by Lucey et al. (1998), which could be due to differences in heat treatment and starter culture.

Lucey et al. (1998) heated skim milk at 80°C for 30 min compared with 95°C for 5 min in the present study, which could affect the gelation properties and pH gelation value. In addition, it has been reported that EPS produced by starter culture could elevate the pH gelation value, which could be attributed to the EPS

Table 3. Disulfide bond content ($\mu\text{mol/g}$ of protein; means $\pm$ SD) of unheated and heated milk protein samples with different casein to whey protein ratios

Treatment	Casein to whey protein ratio			
	4:1 (Control)	3:1	2:1	1:1
Unheated (25°C)	$0.20 \pm 0.01^{\text{d,B}}$	$0.23 \pm 0.01^{\text{c,B}}$	$0.27 \pm 0.02^{\text{b,B}}$	$0.33 \pm 0.03^{\text{a,B}}$
Heated (95°C)	$14.28 \pm 1.47^{\text{d,A}}$	$20.07 \pm 1.51^{\text{c,A}}$	$25.25 \pm 1.07^{\text{b,A}}$	$27.78 \pm 1.24^{\text{a,A}}$

^{a-d}Means within a row with different letters are significantly different ($P < 0.05$).

^{A,B}Means within a column with different letters are significantly different ($P < 0.05$).

Table 4. Surface hydrophobicity (S_0 , unitless; means $\pm$ SD) changes of unheated and heated milk protein samples with different casein to whey protein ratios

Treatment	Casein to whey protein ratio			
	4:1 (Control)	3:1	2:1	1:1
Unheated (25°C)	1,731.72 $\pm$ 17.54 ^{a,B}	1,567.23 $\pm$ 18.38 ^{b,B}	1,416.24 $\pm$ 19.70 ^{c,B}	1,351.32 $\pm$ 17.64 ^{d,B}
Heated (95°C)	2,178.01 $\pm$ 28.31 ^{d,A}	2,565.02 $\pm$ 14.27 ^{c,A}	2,720.05 $\pm$ 12.08 ^{b,A}	2,898.25 $\pm$ 20.07 ^{a,A}

^{a-d}Means within a row with different letters are significantly different ($P < 0.05$).

^{A,B}Means within a column with different letters are significantly different ($P < 0.05$).

charge to facilitate the interaction between protein and polysaccharides, and EPS steric hindrance to induce casein micelles to interact with each other at higher pH (Mende et al., 2013).

In addition, the TS content of the milk base (Table 1) decreased with WPC addition, which meant the contents of lactose and minerals in the milk base decreased with constant total protein concentration. However, yogurt properties still improved with the decrease in TS contents, so this is likely not an important factor affecting yogurt properties.

With an increase in CN:WP ratio, more hydrophobic groups were exposed and more disulfide bonds were produced after heat treatment, as shown in Tables 3 and 4, which was consistent with the result of Nicolai et al. (2011). Whey proteins unfold and expose inner hydrophobic groups when heated at temperatures above 65°C (Croguennec et al., 2004) and tend to interact with themselves or κ -casein to form heat-induced polymers (Jang and Swaisgood, 1990). Denatured β -LG molecules might be involved in thiol oxidation reactions and in intermolecular interchange reactions at higher temperatures (Sava et al., 2005). Therefore, a high concentration of whey proteins could provide large amounts of free thiol groups of β -LG to form disulfide bonds, and high temperature could induce β -LG structure changes, which are mainly responsible for the altered rheological properties and structures of low-fat acid gels; α -LA is significantly involved in formation these complexes as well (Law et al., 1994; Noh et al., 1989).

The aggregation of protein could be hampered and rheological properties could become weak if CN:WP ratios increase, because the excess casein micelles that were not covered adequately with whey protein might not be aggregated by non-disulfide bonds or not aggregated. Low heating intensity could increase formation of polar bonds between proteins but weaken the hydrophobic interaction in milk with a high portion of whey protein. Appropriate CN:WP ratio in milk samples and heat treatment are required to provide sufficient disulfide bonds for casein-whey protein aggregates and whey protein aggregates, which could be key to gelation.

CONCLUSIONS

Changing CN:WP ratio combined with a constant total protein content could dramatically affect the physical and rheological properties of low-fat yogurt. As the CN:WP ratio decreased, yield stress and G' of the obtained yogurt increased, fermentation time was decreased, and microstructure became more homogeneous. When the CN:WP ratio was 2:1 or 1:1, the yogurt gel possessed better rheological properties with the highest G' value and yield point, the shortest gelation period, and a compact network with more disulfide bonds and hydrophobic interactions. Therefore, the CN:WP ratio can be optimized to improve the quality of low-fat yogurt in practical manufacture. In addition, proper heat treatment, which affects the denaturation and interaction of milk proteins, was an important contributor to gel formation of low-fat yogurt.

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